# DEVELOPMENT OF BIO-FORMULA COMPLEX FROM AGRICULTURAL BY-PRODUCTS AND KIMCHI AS MEAL SUPPLEMENT FOR PIGS

Soo Kyung Park, Hyun Jung Bae, Mi Young Kang

Department of Animal Science, Kyungpook National University, Daegu 702-701, South Korea

* Corresponding author (phone/fax: +82-53-950-6235; e-mail: mykang@knu.ac.kr)

***Abstract*–the effect of bio-formula complex developed using agricultural by-products and probiotics on the quality of pork in fattening pigs was determined. The isolated microorganisms from kimchi, identified as *Leuconostoc mesenteroides* subsp. *mesenteroides, Leuconostoc mesenteroides* subsp. *cremoris, Lactobacillus plantarum,* and *Pediococcus pentosaceus* based on their 16S rDNA sequences and growth rates, showed antibacterial activity against *E. coli*. A total of 60 pigs (70 days old, 28 kg) were used in the feeding trial. They were fed with either commercial mixed feed or bio-formula complex feed produced from agricultural by-products with 1% probiotics isolated from kimchi. The antiviral activity and immune response efficacy of the probiotics were investigated. The physicochemical properties and sensory characteristics of pork from pigs fed with the experimental diets were also analyzed. The results showed that the developed bio-formula complex improved the quality of pork.**

***Index Terms*** – bio-formula complex, fermented feed, kimchi, probiotics

## I. INTRODUCTION

With the recent increase in meat consumption, the demand for high-quality meat has also increased. For this reason, it is necessary to produce high-quality pork to satisfy consumers' wants. Antibiotics are largely used to help promote blastoprolepsis in domestic animals and prevent digestive organ disorder like diarrhea. However, improper and prolonged usage of antibiotics causes tolerance. In addition, the antibiotics residue in the animals is also a problem (Jang, 1999). Some European countries prohibit the use of antibiotic material to animals like fattening pigs (Park, 1999). For these reasons, the use of feed additives is being considered as a substitute for antibiotics (Kim, 2001). Probiotics settles down in the host. They restrict the growth of harmful bacteria and create acid, hydrogen peroxide or bacteriocin which could inhibit pathogens (antimicrobial treatment). It has been reported that probiotics prevent digestive organ disease (Tannock et al., 1988), increase immune response efficacy (Kimura et al., 1997) and reduce the antimutation or anticancer activity (Fuller and Gibson, 1997). Especially, probiotic lactic acid bacteria are frequently used in producing fermented milk products and useful probiotics in some foods (Hood et al., 1988). The lactic acid bacteria from kimchi, a Korean traditional food, produce antimicrobial materials such as organic acid and bacteriocin. They inhibit the growth of harmful bacteria and produce some flavor compounds. Previous studies revealed that codominant lactic acid bacteria found from kimchi were *Lactobacillus, Leuconostoc , Pediococcus, Weissella*. It has been reported that by using 16S rDNA analysis and RAPD-PCR identification method (Kim and Chun, 2005), *Weissella koreensis* (42.6~82%) was found to be the major bacteria in kimchi. According to researchers, the presence of codominant lactic acid bacteria in kimchi varies depending on the manufacturing place, food ingredients and ripening temperature. Moreover, microorganisms separated from kimchi were reported to inhibit AI virus. Hence, kimchi became the research focus in food and animal feed industries. In some areas, *Weissella* sp. has been used in foods and as feed additives (Lee, 2006). As a Korean traditional fermented food, kimchi is safe for consumption and may have the potential to become a fermentation starter for animal feeds in the future.

## II. MATERIALS AND METHODS

### *A.* Preparation of bio-formula complex from food and agricultural by-products for the production of fermented feed

Seven agricultural and food by-products, such as rice bran, barely bran, ramen crumbs, dried leftover food, ground corn, soybean cake and hwangtoh, were used as raw materials for the preparation of bio-formula complex. In addition, the experiment carried out nitrogen coefficiency and standardization with lysine and glucose as components of the feeding mixture (Table 1).

Table 1. Feeding mixture component for solid fermented feed.

| Material | Mixing rate (%) | Calorie (Kcal) |
|---|---|---|
| Rice bran | 22.68 | 74.63 |
| Barely bran | 22.68 | 63.1 |
| Ramen crumbs | 22.68 | 78.42 |
| Dried leftover food | 3 | 12.3 |
| Groung corn | 20.57 | 72.33 |
| Soybean cake | 8.31 | 26.06 |
| Hwangtoh | 0.59 | - |
| Lysine | 0.15 | 0.6 |
| Glucose | 0.15 | 0.6 |
| total | 100 | 344.41 |

*B.* Identification of microorganisms isolated from *kimchi*

Two grams of kimchi, purchased from a local market, was blended in an aseptic condition and diluted with sterile distilled water. The mixture was plated in MRS agar plate at 35°C for 24 hr. A well-isolated colony of lactic acid bacteria was subjected to 16S rDNA analysis. The 16S rDNA sequence was analyzed according to Thompson method. The 16S rDNA sequence was compared with ribosomal DNA sequence in GENEBANK using a BLASTIN program. The homology of sequence was analyzed using a Mega 2 program and Clustal X.

*C.* Preparation of probiotics and fermented feed

A culture medium containing 2.5% decocted medicinal herbs (cinnamon, Korean angelica root, licorice), 5% black sugar, P (0.1%), Ca (0.04%) was prepared. Four kinds of the isolated cultures were added to the medium and incubated for 18 hr at 35°C. The prepared probiotics (1%) was added to the feeding mixture and incubated for 8 hr at 35°C. The resulting fermented feed mixture was used as feeds to fattening pigs.

*D.* Analysis of the nutrient content of the feeds

The nutrient contents and energy efficiency of the commercial mixed feed and probiotics fermented feed were analyzed and compared.

*E.* Feeding trial of the probiotics fermented feeds

A total of 60 pigs (70 days old, 28kg) were used in the feeding trial. The animals were divided into two groups and fed with either a commercial mixed feed or the bio-formula complex feed produced from agricultural by-products with 1% probiotics isolated from kimchi.

## III. RESULTS AND DISCUSSION

Identification of lactic acid bacteria isolated from kimchi

The isolated microorganisms from kimchi were identified as *Leuconostoc mesenteroides* subsp. *mesenteroides*, *Leuconostoc mesenteroides subsp. cremoris*, *Lactobacillus plantarum,* and *Pediococcus pentosaceus* based on their 16S rDNA sequences and growth rate.

Antimicrobial activity

To measure the antimicrobial activity, 4 different isolated cultures were cultured with *Escherichia coli* ATCC 8739 in MRS broth and EMB agar plate. The total counts of *E. coli* in MRS broth decreased after 9 hr (Fig. 4).

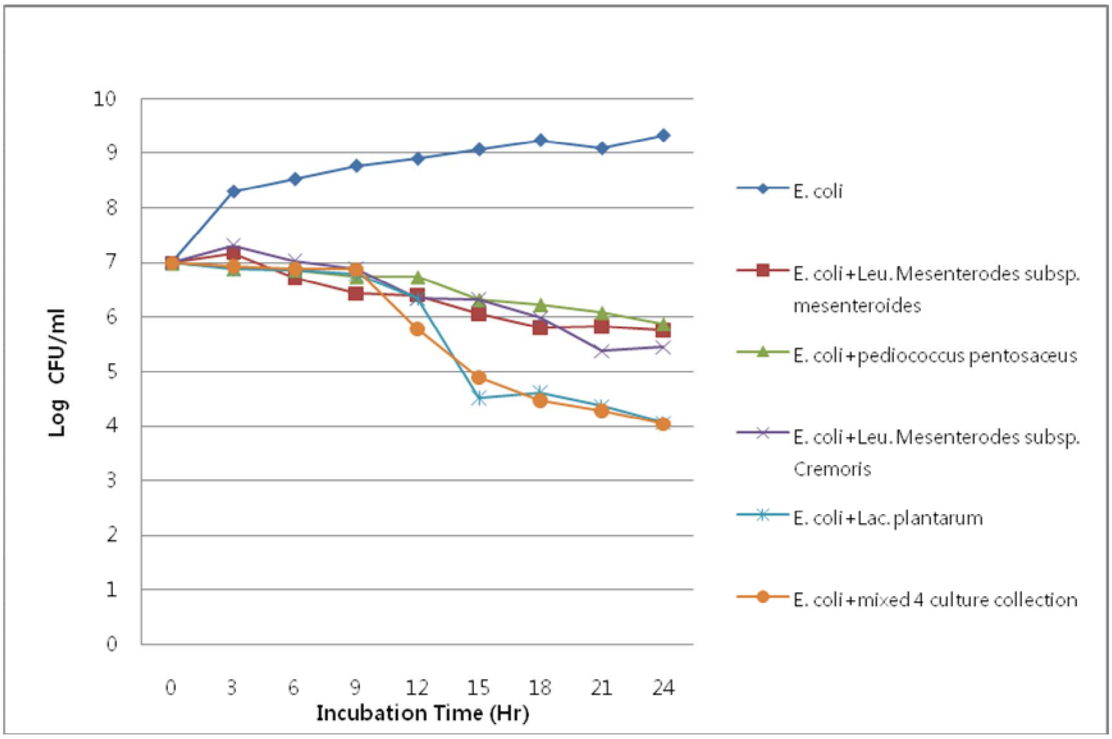


Fig 4. Antimicrobial activity of *L. mesenteroides* subsp. *mesenteroides*, *L. mesenteroides* subsp. *cremoris*, *L. plantarum*, *Pediococcus pentosaceus* and mixture of the 4 isolated bacteria from kimchi against *E. coli* ATCC 8739 in EMB agar plate.

Analysis of the fermented feed's nutritional level

Table 2 shows the nutrition level of the solid fermented feed. In comparison with the commercial feed, the fermented feed contained higher levels of crude fat, crude ash, crude fiber, Ca and P and lower crude protein content. The energy per kilo of feed and pepsin digestibility were slightly lower in fermented feeds than in commercial ones (Table3).

Table 2. Nutrient contents (%) of commercial and fermented feeds

| | Moisture | Crude protein | Crude fat | Crude fiber | Crude ash | Ca | P |
|---|---|---|---|---|---|---|---|
| (A) | 13.3 | 18.1 | 4,17 | 3.58 | 5.36 | 1.04 | 0.55 |
| (B) | 17.1 | 16.6 | 6.27 | 4.49 | 6.30 | 1.61 | 0.66 |

(A), commercial mixed feed; (B), fermented feed

Table 3. Energy level and pepsin digestibility of commercial and fermented feeds

| | Energy (ME Kcal/Kg) | Pepsin Digestibility(%) |
|---|---|---|
| (A) | 3,916 | 89.66 |
| (B) | 3,764 | 82.44 |

(A), commercial mixed feed; (B), fermented feed

Antiviral activity and immune response efficacy of solid fermented feed

A total of 60 pigs (70 days old, 28kg) were used in the feeding trial. Half of them were fed with commercial mixed feed and the other half were given with bio-formula complex feed from food by-products containing 1% probiotics isolated from kimchi. During the feeding trial, some animals died from respiratory disease. However, the commercial mixed feed trial group (A) showed higher death rate than probiotic fermented feed group (B). Eight fattening pigs died and the death ratio was 27% in group A while group B showed 17% death ratio with 4 dead animals (Table 4). The animal death in group B occurred 15 days after the first death occurrence in group A. Moreover, the death occurrence in group A stopped 20 days earlier than that of group B. Considering these facts, the antiviral activity and immune response efficacy of solid fermented feed were effective.

Table 4. The number of dead pigs due to respiratory disease during feeding trial

| Death occurrence due to respiratory disease | | (A) | (B) |
|---|---|---|---|
| Month | Date | | |
| 10 | 5 | ● | |
| | ↕ | ↕ | ↕ |
| | 20 | | ● |
| | 21 | | ● |
| | 24 | ● | ● |
| | 25 | ● | |
| | 27 | ● | |
| | 28 | | ● |
| | 29 | | |
| | 31 | ● | |
| 11 | 1 | | |
| | ↕ | ↕ | ↕ |
| | 14 | ● | |
| | 18 | ● | |
| | 21 | ● | |
| Total | | 8 heads | 4 heads |

(A), commercial mixed feed group; (B), fermented feed group

Physicochemical properties and sensory evaluation of pork

Weight reduction after heating

The chuck roll (6g) and pork belly (6g) samples were placed in separate zipper bags. Heat treatment was applied for 10 min in a water bath and the samples were cooled for 30 min at room temperature. The water loss was determined using the following formula:

$$\text{Water loss rate (\%)} = \frac{\text{Sample weight before heating(g)} - \text{Sample weight after heating}}{\text{sample weight before heating(g)}} \times 100$$

Table 5. Weight reduction in pork samples after heating

| | Sample weight before heating (g) | | Sample weight after heating (g) | | Water loss (g) | | Water loss rate (%) | |
|---|---|---|---|---|---|---|---|---|
| | (A) | (B) | (A) | (B) | (A) | (B) | (A) | (B) |
| Chuck roll | 5.79±0.17 | 5.9±0.05 | 3.3±0.08 | 3.49±0.18 | 2.5±0.09 | 2.42±0.14 | 43.02±0.37 | 40.97±2.61 |
| Pork belly | 5.92±0.04 | 6.08±0.01 | 4.39±0.04 | 4.7±0.02 | 1.53±0.03 | 1.39±0.01 | 25.89±0.4 | 22.85±0.20 |

(A), commercial mixed feed group; (B), fermented feed group pH

The fat and fascia of the chuck roll and pork belly samples were removed. Three grams from each meat sample was minced evenly and added with 27 ml distilled water (pH 6.8). The sample mixture was homogenized for 30 sec using a homogenizer and the pH was determined using a pH meter.

Table 6. pH of the meat samples

| | Treatment (A) | Treatment (B) |
|---|---|---|
| Chuck roll | 6.09±0.01 | 5.78±0.01 |
| Pork belly | 5.94±0.006 | 5.84±0.003 |

(A), commercial mixed feed group; (B), fermented feed group

Sensory evaluation

The sensory quality pork was evaluated by 8 graduate students using a 9-point hedonic scale (1: extremely bad, 9: extremely good). For cooking methods, boiling and grilling were employed. Group (B) showed higher sensory score for aroma and flavor than group A.

Table 7. Sensory scores of pork from pigs fed with dietary commercial and fermented feeds

| | (A) | | (B) | |
|---|---|---|---|---|
| | Aroma | Flavor | Aroma | Flavor |
| Chuck roll | 4.8 | 5 | 4.9 | 5.35 |
| Pork belly | 5.2 | 5.15 | 5.4 | 5.3 |

Aroma (1: extremely bad, 9: extremely good); flavor (1: extremely bad, 9: extremely good)
(A), commercial mixed feed group; (B), fermented feed group

## IV. CONCLUSION

The present study illustrate that the bio-formula complex feeds developed using agricultural by-products and probiotics have high antiviral activity and immune response efficacy when fed to fattening pigs. Moreover, this bio-formula complex feed improved the physicochemical properties and sensory quality of pork. Hence, the utilization of probiotics and agricultural by-products may be useful in the production of meal supplements for pigs in order to enhance the quality of pork.

## ACKNOWLEDGEMENT

This study is encouraged by project of Youngju City. Research funds are provided from Youngju City.

## REFERENCES

Hood, S. K. & Zottola, E. A. (1998). Effect of low pH on the ability of Lactobacillus acidophilus to survive and adhere to human intestinal cells. *Journal of Food Science*, 53, 1541-1516.

Kim, J. H., Kim, C. H., & Go, Y. D. (2001). The effect of productivity and economic feasibility at added economics in animal’s feed. *Supports in Animal Resources Science Field Animal Resources, KOSEF*, 43, 369-380.

Kim, M. G. & Chun, J. S. (2005). Bacterial community structure in kimchi, a Korean fermented vegetable food, as revealed by 16S rDNA gene analysis. *International Journal of Food Microbiology*, 103, 91-96.

Kimura, K., McCartney A. L., McConell M. A., & Tannock G. W. (1997). Analysis of fecal population of bifidobacteria and lactobacilli and investigation of immunological response of their human hosts to the predominant strains. *Applied and Environmental Microbiology*, 63, 3394-3398.

Park, J. Y. (1999). Introduce the contraindication of add a antibiotics in animal's feed in EU. Support in The journal of Korean veterinary medical association 35, 198-205.

Tannock G. W., Crichton C., Welling G. W. Koopman J. P., & Midtvedt, T. (1998). Reconstitution of the gastrointestinal microflora of lactobacillus-free mice. *Applied and Environmental Microbiology*, 54, 2971-2975.